ABSTRACT

Background: Dyslipidemia in chronic renal insufficiency is one of the factors contributing to atherosclerosis and cardiovascular abnormalities. Changes in lipid status and their relation to severity of the disease is an active area of research, both in predialysis chronic kidney disease (CKD) patients and in patients on maintenance hemodialysis (HD).

Aim and objectives: We have undertaken this study with a view of analyzing the abnormal lipid profile pattern including Lipoprotein(a) or Lp(a), total cholesterol, triglycerides, Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL), in all stages of predialysis CKD patients and their correlation with GFR. We have also attempted to study the abnormal lipoprotein metabolism in patients on maintenance hemodialysis (CKD stage V).

Materials and methods: A total of 100 non diabetic subjects were further divided into 4 subgroups based on the GFR. Group 1 comprising of healthy controls, group 2 of stage I and II CKD patients, group 3 of stage III and IV CKD patients and group 4 of stage V CKD patients on hemodialysis. Fasting blood samples were collected and analyzed for Lp(a), total cholesterol, triglycerides and HDL levels. LDL, GFR and BMI were the calculated parameters. GFR was calculated using MDRD formula and LDL by Freidwald's formula.

Statistical analysis: Statistical analysis was performed using SPSS version 16. One-way analysis of variance (ANOVA) and Pearson's Correlation coefficient and Regression equation were done.

Results: There is an increase in serum Lp(a) levels in all stages of CKD patients but significant increase is seen in groups 3 and 4 patients compared to that of the control group 1 (P<0.01). There is a significant rise in serum triglyceride levels in group 3 patients from that of the controls (P<0.01). There is a decrease in total cholesterol levels in group 4 patients compared to the controls (P<0.01). On comparison of serum LDL levels of controls with the other 3 groups, there is a significant elevation in groups 3 and 4 patients (P<0.01). There is no significant change in the serum HDL levels among the 4 groups. A negative significant correlation has been observed between Lp(a) and GFR.

Conclusion: The abnormal lipoprotein pattern especially Lp(a) seen in chronic renal failure patients may start early and progress with the declining renal function. These dysregulated lipoproteins may contribute to atherosclerosis and cardiovascular disorders which is the leading cause of death in these patients.

Key words: Chronic kidney disease, hemodialysis, Lipoprotein(a), dyslipidemia

INTRODUCTION

Chronic kidney disease (CKD) is associated with early development of atherosclerosis and increased risk of cardiovascular morbidity and mortality which is the leading cause of death among these patients. Alterations in lipid metabolism resulting in abnormal lipoprotein composition and concentration (dyslipidemia) have been noticed in chronic renal insufficiency. This dyslipidemia not only plays an important role in the pathogenesis of atherosclerosis in predialysis CKD patients but also in patients on maintenance hemodialysis, who already have an inherent risk.

There are many specific abnormalities in the lipoprotein metabolism in CKD patients. Among them, increased Lp(a) is an independent risk factor for atherosclerosis and hence a strong predictor of coronary events in chronic renal failure. There is strong evidence that Lp(a) rises very early in renal
Lipoprotein (a) and Dyslipidemia

Lp(a) is an LDL-like lipoprotein consisting of apo(a) which shows a strong homology with plasminogen competing with it for binding to fibrinogen and fibrin. Many studies have shown an elevation in serum Lp(a) levels with decreasing GFR, both in predialysis and hemodialysis CKD patients.

Changes in lipid profile pattern have been identified by many authors who have tried to elucidate the causes and consequences of the dysregulation of lipid metabolism, in all stages of CKD patients. In these patients, the lipid parameters namely serum Lp(a), total cholesterol, triglycerides, LDL (low density lipoprotein) and HDL (high density lipoprotein) cholesterol have been individually studied by many authors and noted for potential complications. In our study we have analyzed the levels of the above said parameters in different stages of predialysis CKD patients and stage V CKD patients on maintenance hemodialysis. We have also looked for any potential correlation between their levels and GFR and have attempted to evaluate the significance of all the changes identified.

**MATERIALS AND METHODS**

**Study Design and Patients**

The study was conducted on 100 non diabetic subjects, out of which 75 were CKD patients and 25 were healthy individuals. The CKD patients were further classified based on GFR as per the NKF K/DOQI guidelines. Group 1 included 25 healthy individuals (GFR > 90 ml/min/1.73 m²), group 2 consisted of 25 patients in CKD stages I and II (GFR > 60 ml/min/1.73 m²), group 3 comprised of 25 patients with CKD stages III and IV (GFR of 15 to 59 ml/min/1.73 m²) and group 4 constituted 25 patients with CKD stage V (GFR < 15 ml/min/1.73 m²) on maintenance hemodialysis. The predialysis and hemodialysis CKD patients were selected from the wards and outpatient departments of Vinayaka Missions Hospital, Vinayaka Missions Kirupananda Variparana Medical College and from Nephrology Dept in Salem Poly Clinic, Salem. The study has been approved by the Ethical committee of Vinayaka Missions Kirupananda Varipanada Variparana Medical College and informed consent was obtained from all the subjects. All the relevant demographic data were collected and the subjects were matched for their age and sex. Height and weight were measured to calculate BMI (height/weight²).

Patients (groups 2, 3 and 4) with diabetes, obesity, liver disease and systemic illnesses were excluded from the study. Healthy individuals without diabetes, hypertension, renal disease and any other systemic illnesses were selected for the study and they formed the control group (group 1).

Fasting blood samples were collected from all the patients (groups 2, 3 and 4) and controls (group 1) after an overnight fast. Blood was allowed to clot and serum separated after centrifuging the samples. The sera were used for the analysis of biochemical parameters like fasting glucose, urea and creatinine along with the lipid parameters - Lp(a), total cholesterol, triglycerides, LDL and HDL-cholesterol. In Hemodialysis (HD) patients, predialysis fasting blood samples were obtained.

**Biochemical Methods**

Fasting blood sugar, urea and creatinine levels were estimated using commercially available kits in semiautonoanalyzer – Microlab 300(Merck). Serum Lp(a) levels were determined using latex turbidimetry method - Lp(a)- turbilatex, in semiautonoanalyzer (microlab 300). Other lipid parameters were also analyzed using the same semiautonoanalyzer (microlab 300). Serum total cholesterol and triglycerides were estimated using Innoline kits by endpoint analysis method while HDL was measured using direct enzymatic method (HDLc-D -Labkit). GFR was calculated using Modification of Diet in Renal Disease (MDRD) formula and serum LDL-cholesterol levels were calculated using Friedwald’s formula: LDL cholesterol= Total cholesterol- HDL cholesterol- TGL/5. BMI was calculated using the formula height/weight². Urine samples from the patients and controls were analyzed using Siemen's Multistix 10 SG reagent strips.

**Statistical Analysis**

Statistical analysis was performed using SPSS version 16. One-way analysis of variance (ANOVA) was done to compare the means, Pearson’s
Correlation co-efficient and Regression equation for the variables were done.

RESULTS

A total of 100 age and sex matched subjects were recruited for the study. Among them, 75 were CKD patients (groups 2, 3 and 4) and 25 (group1) belonged to the control population.

The mean and standard errors of the various lipid parameters Lp(a), total cholesterol, triglycerides, LDL and HDL along with BMI were analyzed and their results shown in Table 1. There is an increase in serum Lp(a) levels in all stages of CKD patients with a significant rise in the group 4 hemodialysis patients (57.60 ± 6.64 mg/dl) from that of the controls (17.24 ± 1.97 mg/dl) and group 2 (30.54 ± 4.14 mg/dl) patients (P<0.01). The group 3 subjects (49.55 ± 8.03 mg/dl) also had significantly elevated Lp(a) levels compared to the controls and group 2 patients (P<0.01). There was no significant difference between the controls and group 2 individuals and also among the groups 3 and 4.

Serum triglycerides (TGL) in group 3 patients (162.48 ± 14.17 mg/dl) were significantly higher (P<0.01) than that of the controls (120.64 ± 6.88 mg/dl), group 2 (122.84 ± 9.46 mg/dl) and hemodialysis patients (101.08 ± 7.66 mg/dl). There was no significant change in its levels among the groups 1, 2 and 4. The serum total cholesterol levels showed no significant difference in groups 2(153.08 ± 6.79 mg/dl) and 3 when compared to the control group 1(163.24 ± 7.75 mg/dl). The group 4 hemodialysis patients had total cholesterol levels(116.40 ± 7.76 mg/dl) significantly lower than that of the groups 1, 2 and 3(p<0.01).

The serum LDL levels were significantly lower in the hemodialysis group (55.06 ± 7.03 mg/dl) compared to the controls (96.13 ± 8.02 mg/dl) and patients (84.61 ± 5.84 mg/dl) in group 2 (P<0.01). Group 3 patients (73.58 ± 6.90 mg/dl) also showed a lowering of serum LDL levels from that of the control group1 (P<0.01). The BMI was significantly reduced (P<0.05) in groups 3 (22.24 ± 0.80 mg/dl) and 4(22.12 ± 0.67 mg/dl) compared to that of controls (25.47 ± 0.89 mg/dl). There was no significant change in the serum HDL levels among the 4 groups.

Table 2 shows the correlations of Lp(a), TGL and BMI with GFR, along with their levels of significance. There was a highly significant negative correlation between the serum Lp(a) levels and GFR among the 100 subjects taken for the study(p<0.01), with a correlation coefficient of -0.475. The serum triglyceride levels, didnot show any significant correlation with the GFR (P=0.548). A positive significant correlation was observed between BMI and GFR (p<0.01), with a correlation coefficient of 0.295.

DISCUSSION

Patients with chronic kidney disease (CKD) exhibit alterations in lipoprotein metabolism which might predispose to the development of atherosclerosis and cardiovascular disease (CVD). We have assessed the serum levels of Lp(a) and other lipid parameters namely triglycerides, total cholesterol, LDL and HDL in 4 groups of subjects (group 1-controls, group 2-CKD I & II, group 3- CKD III & IV, group IV-hemodialysis patients).

We have found in our study that the serum Lp(a) levels are significantly increased in predialysis CKD patients (group 3) and in hemodialysis patients (group 4), compared to that of the controls (group 1). Elevated serum Lp(a) levels have frequently been reported in predialysis CKD patients and patients on hemodialysis in patients with nephrotic syndrome. There is also a negative significant correlation present between serum Lp(a) and GFR which shows that Lp(a) levels are influenced by GFR and they tend to rise with the reduction in GFR. This finding of ours correlate with that of Leonardo et al and Kronenberg et al. It has also been found that the Lp(a) levels are elevated in CKD even before the GFR starts to decrease.

Lp(a) is an LDL-like particle having an apolipoprotein (a) which is attached to apolipoprotein B-100 by a disulfide linkage. It is synthesized in the liver, but its sites of catabolism are not clear. The increase in Lp(a) levels in CKD patients could be due to its increased synthesis by the liver or due to its decreased catabolism in kidneys. A significant decrease in Lp(a) concentrations between the ascending aorta and renal vein and the
Identification of apo(a) fragments in urine indicate kidneys' active participation in the degradation of Lp(a). Recent studies have also shown a strong genetic basis for the increase in serum Lp(a) levels in chronic kidney disease. In chronic kidney disease, individuals with low molecular weight (LMW) apo(a) isoforms have been shown to have high serum Lp(a) levels and those with high molecular weight HMW apo(a) isoforms have low levels. In hemodialysis patients, by in vivo turnover studies using stable isotope techniques, Frischmann KE et al. have elucidated that the fractional catabolic rate of the apolipoprotein(a) was significantly reduced resulting in its longer residence time in plasma (9 days) compared to the controls (4.4 days). This decreased clearance could be the result of loss in kidney function, in hemodialysis patients. Malnutrition and inflammation have also been associated with high plasma Lp(a) levels in hemodialysis patients.

There is an increase in serum triglyceride levels in CKD patients, as the GFR declines. Compared to controls, this increase is significant in predialysis group 3 patients, while those in group 2 donot show any significant change. Plasma triglycerides are predominantly present in 2 types of lipoproteins, namely the chylomicrons and VLDL. Hypertriglyceridemia may be due to high production rate of these lipoproteins and a low catabolic rate. Renal insufficiency can cause insulin resistance which in turn promotes hepatic VLDL production and hence elevated triglyceride levels. But the predominant mechanism for increased triglyceride levels in predialysis patients is that of delayed catabolism and hence impaired clearance. The reduced catabolism is due to the decreased activity of 2 endothelium-associated lipoprotein lipases - hepatic lipase and lipoprotein lipase (LPL). There may be down regulation of the LPL and hepatic lipase enzyme gene expressions, contributed in part by secondary hyperparathyroidism. The decrease in lipoprotein lipase activity may be due to the increase in plasma apo C-III levels resulting in a decrease in apo C-II/ apo C-III ratio. Apo C-II is an activator of lipoprotein lipase while apo C-III is a potent inhibitor of LPL and so the increase in apo C-III levels results in inactivation of lipoprotein lipase resulting in reduced triglyceride lipolysis and hence increased triglyceride levels. Another inhibitor of lipoprotein lipase has been identified as pre-β-HDL, whose concentration is found to be elevated in CKD patients. Though many studies have shown hypertriglyceridemia in hemodialysis patients, we did not observe any significant change in this group of patients. This may either be due to the patients receiving carnitine injections, multivitamin supplementations or HMG-CoA inhibitors. These factors could have marginally prevented the rise of serum triglyceride levels in hemodialysis patients. There is some correlation between serum triglycerides and GFR but it is not significant.

There is significant lowering of serum total cholesterol levels in hemodialysis group 4 patients compared to that of controls and other predialysis CKD patients. There is minimal lowering of its levels in predialysis CKD patients compared to the controls, though it is not significant. Many authors have noticed that in hemodialysis patients low serum cholesterol is associated with increased mortality. It appears that many dialysis patients have a condition identified as malnutrition-inflammation complex syndrome (MICS), which is a combination of protein-energy malnutrition and inflammation and is related to poor dialysis outcomes. This MICS leads to a low body mass index, hypocholesterolemia, hypocreatininemia, and hypohomocysteinemia, increasing the risk of death. The hypocholesterolemia is a strong mortality risk factor in dialysis patients and a marker of poor nutritional status. The mechanism by which systemic inflammation and malnutrition may explain this hypocholesterolemia is unclear. A cytokine-mediated acute-phase reaction to acute or chronic inflammation may partially account for the hypocholesterolemia (cholesterol-negative acute phase reactant), in dialysis patients by increasing catabolism and decreasing appetite. In our study, we have also identified a significant reduction in body mass index (BMI) in hemodialysis and group 3 patients, compared to the controls.

We can see a lowering of the LDL levels in group 3 patients and also in the hemodialysis group compared to that of controls. This lowering of LDL
in CKD may be due to the same above said reason-inflammation/malnutrition or due to reduced production of LDL resulting in its near normal levels. The inflammation may change the lipoprotein structure and function by oxidatively modifying low density lipoprotein. In CKD patients there is a relative increase in IDL (intermediate density lipoproteins) and small dense LDL (sdLDL) particles which undergo further modifications like glycation, oxidation and carbamylation, making them highly atherogenic. These modified lipoproteins are in turn taken up by the scavenger receptors on macrophages and vascular smooth muscle cells, which are increased in uremia, favoring the development of atherosclerotic plaques. Though many studies have shown a reduction in HDL level with progression of renal disease, we could not find any such change.

CONCLUSION

Our findings of uremic dyslipidemia are that of increased Lp(a), hypertriglyceridemia, hypocholesterolemia and reduced LDL levels, becoming more evident with declining renal function. This dysregulation of lipid metabolism may contribute to the pathogenesis of atherosclerosis and cardiovascular disease as well as to the progression of renal disease. Further research have to be done to confirm whether early detection and treatment (diet/drug therapy) of this dyslipidemia is quite promising, in the prevention of adverse clinical outcomes in predialysis CKD patients and in those on maintenance hemodialysis.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>Correlation coefficient</th>
<th>Sig. (2-tailed)</th>
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<tbody>
<tr>
<td>Lp(a) and GFR</td>
<td>-.475**</td>
<td>.000(S)</td>
</tr>
<tr>
<td>BMI and GFR</td>
<td>.295**</td>
<td>.003(S)</td>
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<tr>
<td>TGL and GFR</td>
<td>-.061</td>
<td>.548(NS)</td>
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</tbody>
</table>

(NS- Non significant, S- Significant)
* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

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